MINIREVIEW

Inhibitory Glycine Receptors: An Update*⑤

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Strychnine-sensitive glycine receptors (GlyRs) mediate synaptic inhibition in the spinal cord, brainstem, and other regions of the mammalian central nervous system. In this minireview, we summarize our current view of the structure, ligand-binding sites, and chloride channel of these receptors and discuss recently emerging functions of distinct GlyR isoforms. GlyRs not only regulate the excitability of motor and afferent sensory neurons, including pain fibers, but also are involved in the processing of visual and auditory signals. Hence, GlyRs constitute promising targets for the development of therapeutically useful compounds.

The normal functioning of the CNS depends on the balanced interplay of both excitatory and inhibitory neurons. Glutamate is the principal excitatory and GABA and glycine are the major inhibitory neurotransmitters in the adult mammalian CNS. Glycine serves, in addition, as a co-agonist of glutamate at the NMDA subtype of excitatory glutamate receptors.

Glycinergic synapses mediate fast inhibitory neurotransmission mainly in the spinal cord, brainstem, and caudal brain and control a variety of motor and sensory functions, including vision and audition (1). Glycine exerts its inhibitory effects via specific glycine receptors (GlyRs)[2] that are highly enriched in the postsynaptic membrane. Binding of glycine leads to the opening of the GlyR integral anion channel, and the resulting influx of Cl^- ions hyperpolarizes the postsynaptic cell, thereby inhibiting neuronal firing. The alkaloid strychnine antagonizes glycine binding with high affinity and has proven to be a unique tool in radioligand binding studies (2) and affinity purification (3) of GlyRs. Since these original studies, three decades of GlyR research have generated a wealth of genetic, functional, and structural data, which are summarized here.

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⑤ This article contains supplemental Figs. S1 and S2.
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[2] The abbreviations used are: GlyR, glycine receptor; LGIC, ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor; GABA$_A$R, GABA$_A$ receptor; ECD, extracellular domain; TM, transmembrane segment; GluCl, glutamate-gated channel; sIPSC, spontaneous inhibitory postsynaptic current; PGE$_2$, prostaglandin E$_2$; HKPX, hyperekplexia.

Composition of GlyR Proteins

GlyRs are group I ligand-gated ion channels (LGICs) that belong to the Cys loop receptor family, which, in addition, includes nicotinic acetylcholine (nAChR), serotonin type 3 (5-HT$_3$), and the closely related GABA$_A$ (GABA$_A$R) receptors (4, 5). Affinity-purified GlyR preparations contain three polypeptide species (3): the GlyRα (48 kDa) and GlyRβ (58 kDa) subunits and a tightly bound cytosolic scaffolding protein (93 kDa) later named gephyrin (6). Cross-linking experiments showed that purified GlyRs are heteropentameric proteins, similar to the nAChRs in *Torpedo* electric organ and skeletal muscle (7). Their subunit stoichiometry was originally thought to be 3α:2β (7, 8), but more recently, this was revised to 2α:3β (9, 10).

Molecular cloning identified four vertebrate genes (called *Glra1–4*) encoding GlyRα subunits (α1–α4) and a single gene (*Glrb*) encoding the GlyRβ subunit (11–15). All GlyRα subunits display high sequence identity (>80%) (supplemental Fig. S1) and, upon heterologous expression, form functional homomeric glycine-gated channels with properties closely resembling those of GlyRs *in vivo* (4, 5). By photoaffinity labeling with [³H]strychnine, the GlyRα subunits were shown to possess critical determinants of ligand binding (16). GlyRβ displays significant sequence differences compared with the α subunits (<50% identity) (supplemental Fig. S1) and does not generate functional receptors when expressed alone (11) but is retained in the endoplasmic reticulum (17). However, the GlyRβ subunit is more than simply a structural subunit because it contributes to agonist binding (9) and has an essential role in the intracellular trafficking and synaptic clustering of postsynaptic GlyRs (18, 19). Its extended cytoplasmic loop region binds to the postsynaptic scaffolding protein gephyrin with high affinity (18, 20) and interacts with Vps35 and neurobeachin, proteins implicated in intracellular membrane protein transport (21).

The overall topology of GlyR subunits is shared with that of other group I LGIC proteins (22). All of these polypeptides contain a large N-terminal extracellular domain (ECD), four transmembrane segments (TM1–TM4), a long intracellular loop connecting TM3 and TM4, and a short extracellular C terminus. Sequence homologies are particularly high within the transmembrane segments and a conserved disulfide-bonded motif of 15 amino acids ("Cys loop") located in the ECD. Although the number of GlyR subunit genes ($n = 5$) appears to be modest compared with those found in the mammalian nAChR ($n = 17$) and GABA$_A$R ($n = 20$) families, alternative splicing of exons encoding segments of the extracellular N-terminal or intracellular loop regions further extends GlyR subunit heterogeneity (4, 5, 23). In the case of GlyRα2, splice variants in the ECD have been shown to differ in agonist efficacies (24), and intracellular loop variants of GlyRα3 have been found to differ in receptor desensitization (25) and subcellular targeting (26). Furthermore, post-transcriptional editing of a minor fraction of the GlyRα3 mRNA at a single nucleotide position has been reported to result in a gain-of-function receptor (27). This editing reaction leads to the substitution of proline 185
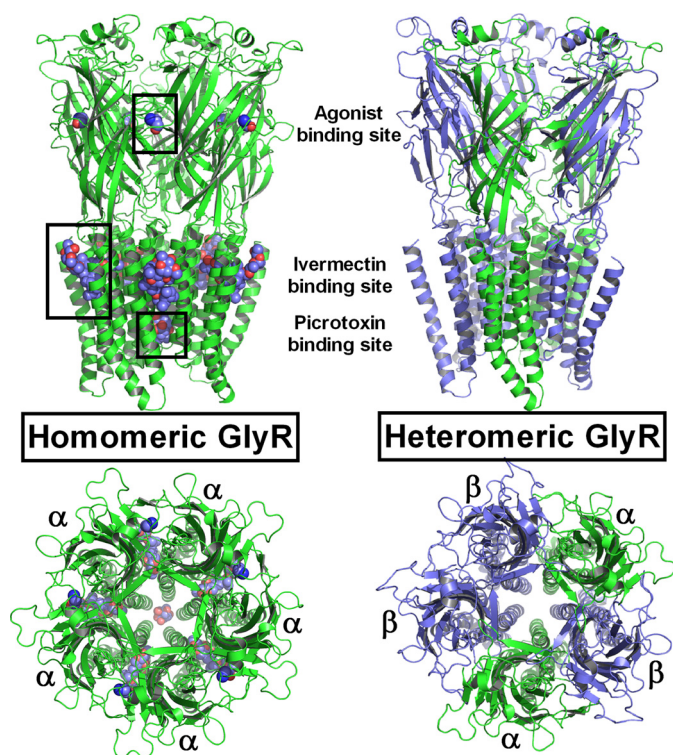
FIGURE 1. **Structures of homomeric α1- and heteromeric α1β-GlyRs modeled using GluCl as a template.** *Left,* α1-GlyR shown with five glycine and ivermectin molecules each and a single picrotoxin molecule bound (ligands depicted as van der Waals *spheres*). *Right,* unliganded heteromeric α1β-GlyR with the different interfaces indicated.

with leucine in the ECD and increases the apparent agonist affinity of α3-GlyRs.

Structure of the GlyR and Its Extracellular Binding Sites

An atomic resolution structure of a GlyR is not available yet despite efforts to obtain suitable amounts of purified protein for structural studies (28). Therefore, structural information has been inferred from other Cys loop receptors or bacterial homologs of the group I LGIC family. Electron microscopy data collected from the muscle-type nAChR from *Torpedo* (29) and the coordinates of the acetylcholine-binding protein AChBP, a soluble homopentameric protein that is structurally homologous to the ECD of group I LGICs (30), have been widely used to model the ECD of many different receptors, including the GlyR (9, 31, 32). Recently, the first crystal structure of an anion-conducting Cys loop receptor, the glutamate-gated channel (GluCl) from *Caenorhabditis elegans*, was solved at atomic resolution (33). GluCl is a close homolog (up to 43% sequence identity) (supplemental Fig. S1) of GlyR subunits and also contains their hallmark feature, the conserved disulfide bridge in loop C. Therefore, GluCl-based models of GlyRs, as shown in Fig. 1, are considered highly reliable templates for ligand binding studies and the rational design of novel selective drugs.

Agonists and competitive antagonists are known to bind to the ECD at the interfaces of two adjacent subunits (4, 5, 22). The GlyR binding pocket is formed by distinct "loop regions" of the principal ($+$) and complementary ($-$) subunit surfaces (Fig. 2). Residues whose substitution affects agonist and antagonist binding have been identified in loops D, B, E, and C (4, 5, 31). In

addition, the disulfide bond between α1Cys-198 and α1Cys-209 constraining loop C appears to be critical for both cell surface expression and ligand binding (34). The binding determinants of the β subunit are less well studied; two GlyRβ residues (Arg-86 and Glu-180, equivalent to α1Arg-65 and α1Glu-157, respectively) (Fig. 2) have been found to influence ligand binding to heteromeric α1β-GlyRs (9). Docking of the agonist glycine and the antagonist strychnine into homology models of the α1-α1 and α1-β interfaces resulted in complex structures that agreed well with previous mutational data (9). However, the properties of the β-β interface had remained enigmatic, as GlyRβ alone does not form functional channels. A novel mutagenesis strategy guided by homology modeling has recently allowed reproduction of all potential agonist-binding sites (α-β, β-α, and β-β) present in heteromeric GlyRs by single-subunit expression (35). Although fully functional, the resulting β-β binding site displayed the most distinct pharmacological profile toward a range of agonists and modulators tested, indicating that it might be selectively targeted to modulate the activity of synaptic GlyRs.

The ECD of GlyRs binds not only agonists and competitive antagonists but also compounds that allosterically modulate the agonist responses of different LGICs. Neurosteroids, general anesthetics, and ethanol potentiate GlyR currents, whereas the divalent cation Zn^{2+}, tropeines, and endogenous cannabinoids, such as anandamide and 2-arachidonylglycerol, have both potentiating (at low concentrations) and inhibitory (at high concentrations) effects. Because of their potential therapeutic importance, the binding sites and isoform selectivity of these modulators have been intensely investigated (4, 5, 31, 36), and their *in vivo* roles have been studied in transgenic and knock-in mouse models (37, 38). Zn^{2+} and tropeines bind within or in the vicinity of the extracellular agonist-binding sites (39, 40). In contrast, hydrophobic modulators, such as neurosteroids and endocannabinoids (41) and anesthetics and ethanol (42), all appear to act by binding to the transmembrane domain (see below), although residues located in the ECD and/or the large intracellular loop may contribute to current modulation (43, 44). Additionally, the principal excitatory transmitter glutamate has been reported to allosterically potentiate native and recombinant GlyRs; this may be important for balancing excitation and inhibition in the CNS (45).

GlyR Chloride Channel

The transmembrane domain of GlyR subunits and other group I LGICs consists of a four-α-helix bundle, in which the transmembrane segments are arranged in a clockwise order due to multiple interactions between specific hydrophobic residues that are essential for proper receptor assembly (46). TM1-TM2 and TM2-TM3 are connected by short loops, whereas TM3 is linked to TM4 by long intracellular loops that contain phosphorylation and ubiquitination sites as well as binding motifs for interacting proteins. The amphiphilic TM2 forms the ion channel; to facilitate comparisons between different LGICs, its residues are often numbered from 1′ to 19′, with position 1′ corresponding to the cytoplasmic N-terminal end and residue 19′ to the extracellular C-terminal end. Substituted cysteine accessibility experiments disclosed that Gly-2′, Thr-6′,
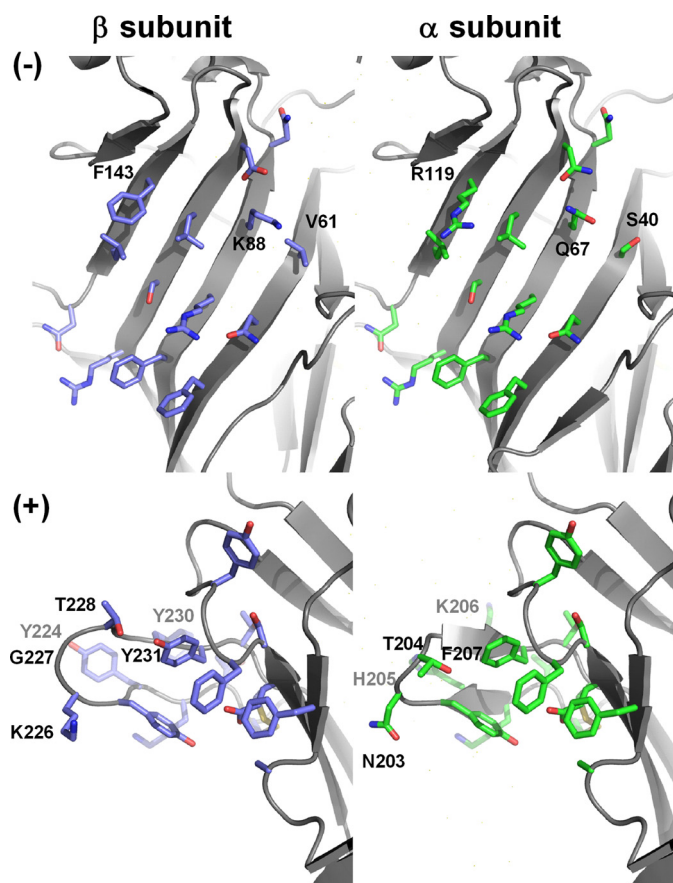
FIGURE 2. **Models of the principal (+) and complementary (−) surfaces of the GlyRα1 and GlyRβ agonist-binding regions.** In heteromeric GlyRs, the + and − surfaces of the α1 and β subunit ECDs generate non-equivalent agonist-binding sites at the α-β, β-α, or β-β interface, which are all functional. Note the significant sequence divergence (up to eight substitutions) between α1 and β, in particular within loop C.

and Arg-19′ line the pore of the α1-GlyR (47). Additional pore-lining residues were inferred by sequence comparison with other LGICs and include Thr-7′, Leu-9′, Thr-10′, Thr-13′, Ser-16′, and Gly-17′ (48). A model of the pore domain of GlyRs based on the recently determined structure of GluCl confirms that most of these residues are indeed facing the channel lumen (supplemental Fig. S2).

Agonist binding to the ECD of GlyRs triggers the opening of the anion-selective channel spanning the plasma membrane. Extensive work on different LGICs has demonstrated that the conformational changes within the five subunits induced upon agonist binding are consistent with an allosteric transition model that predicts rotational movements of the TM2 segments around about position 9′ (29). This model was recently validated by comparing the structures of two bacterial LGIC homologs crystallized in the open and closed states, respectively (49) and is likely to similarly apply to the GlyR. Residues located at the ends or outside of TM2 have been shown to participate in the gating process, such as the GlyRα1 residues Arg-271, located in the M2-M3 linker (34), and Lys-276, a pore-facing residue at the extracellular end of TM2 (50). Presumably, these and other residues that reside at the interface between the ECD and transmembrane domain are essential for coupling the

conformational changes occurring in the ECD upon agonist binding to channel opening (4, 5, 51).

The GlyR channel is largely anion-selective but has been reported to also permeate Na^+ and K^+ ions, albeit at low efficiency (52). GlyRs display a permeability sequence of $SCN^- > NO_3^- > I^- > Br^- > Cl^-$, which is inversely correlated to the hydration energies of these ions, implying that removal of hydration water molecules facilitates ion permeation (53). A "ring of charges," consisting of arginine residues (Arg-0′) in TM2, is thought to concentrate permeating chloride ions and to repel oppositely charged cations. Consistent with this view, the subunits of cation-conducting nAChRs have a negatively charged residue (−Glu-1′) at the equivalent position, and a respective substitution (A−1′E) was found to convert the GlyR into a cation-selective receptor (52). Residues located at the extracellular ends of the TM2 segments and in the adjacent loops (Arg-19′ and Lys-24′) provide additional rings of charge, whose substitution also reduced the unitary Cl^- conductance (54). Besides these positively charged residues, a conserved proline at position 2′ appears to play an important role in determining the selectivity and functional properties of homomeric GlyR channels (55); its deletion increased the pore size of the α1-GlyR and reduced its anion/cation permeability ratio (56). The new GluCl structure shows that this proline residue is located at the narrowest point of the channel and thus directly restricts ion flow (supplemental Fig. S2).

The GlyR channel is blocked by a number of non-competitive antagonists, such as picrotoxin, ginkgolide B, and cyanotriphenyl borate (4, 5). Previous mutagenesis data had indicated that picrotoxin occludes the channel by binding to pore-exposed residues of TM2 (57), although other sites of interaction have also been proposed (58, 59). More recently, Pro-2′ and Thr-6′ of TM2 were found to constitute important determinants of picrotoxin block (60). The crystal structure obtained from the GluCl-picrotoxin complex confirmed this assignment by showing that these residues constitute the binding site for this open channel blocker (33). Furthermore, it supports the idea that the low inhibitory potency of picrotoxin observed at heteromeric compared with homomeric GlyRs (57) is due to steric hindrance by the bulky Phe-282 side chain extending from TM2 of GlyRβ (Fig. 3).

Several hydrophobic modulators of the GlyR are thought to interact with external sites of the transmembrane domain (42, 61). These include neurosteroids, general anesthetics, and alcohols, which all potentiate GlyR function (31). Early mutagenesis experiments had identified a common intrasubunit binding site for these compounds in both GlyRs and $GABA_A$Rs (42), which, because of its predicted size and shape, was termed "big cavity" (62). Within this cavity, the polar residues Ser-267 and Thr-264 located in TM2 and TM3 were shown to influence the enhancing effect of volatile anesthetics and high dose ethanol in GlyRs (42). Recently, the crystal structures of complexes between a bacterial LGIC ortholog from *Gloeobacter violaceus* (GLIC) and two anesthetics (propofol and desflurane) have been solved (63). These structures nicely confirmed the concept of a common intrasubunit binding site in group I LGICs, which preexists in the GLIC apo structure in the upper part of the transmembrane domain of each monomer and recruits anesthetics
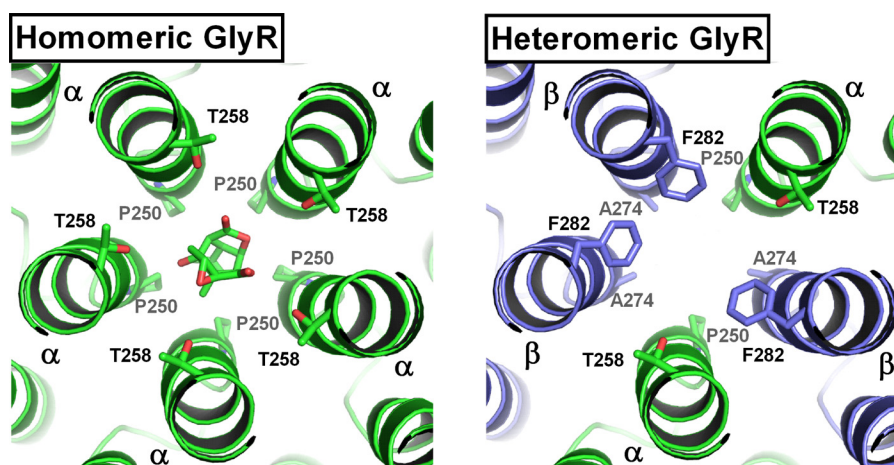
FIGURE 3. **Transmembrane domains of homo- and heteromeric GlyRs.** *Left*, picrotoxin bound in the chloride channel of the homomeric α1-GlyR. Binding determinants include five Pro-250 and Thr-258 residues each. *Right*, in the α1β-GlyR, three of the Thr-258 residues are replaced by Phe-282, and three of the Pro-250 residues by Ala-274 of the β subunit. These substitutions explain the $\sim$50-fold lower affinity of picrotoxin for heteromeric GlyRs.

via van der Waal interactions. This binding may displace crucial lipids and thereby positively or negatively influence channel gating (63). In addition to the big cavity, a second intramembrane site located at the interfaces between adjacent subunits appears to be crucial for potentiation by hydrophobic modulators. The existence of an intersubunit binding site for general anesthetics was first shown in GABA$_A$Rs by photoaffinity labeling with etomidate (64). In GlyRs, this site is targeted by the antihelminthic ivermectin, which allosterically activates both GlyR and GluCl currents. The recent crystal structure of the GluCl-ivermectin complex (33) delineates ivermectin bound at the interface between TM3 from the principal ($+$) and TM1 from the complementary ($-$) subunits (Fig. 1). Furthermore, it shows that ivermectin stabilizes the open state of GluCl by inducing conformational changes in both the transmembrane domain and ECD. Although most of the ivermectin-binding residues of GluCl appear to be conserved in GlyRs, the GlyR binding site for ivermectin has been suggested to differ (65) and hence should be further challenged experimentally.

Roles of GlyR Isoforms in the Mammalian CNS

The physiological functions of the different GlyR isoforms have been incompletely analyzed due to the lack of subtype-specific antagonists. Our present picture of GlyR subtypes is based primarily on immunocytochemical studies and the analysis of knock-out mice.

α1-GlyRs—GlyRs containing the α1 subunit represent the predominant adult GlyR isoform, and heteromeric α1β receptors account for the majority of synaptically localized GlyRs in the mammalian CNS. The importance of the GlyRα1 subunit is underlined by the existence of various disease mutations in the *Glra1* genes of different mammalian species, including mouse, cattle, and human (5). The *spasmodic* mouse carries a substitution at position 52 (A52S) of *Glra1* that reduces the agonist binding affinity (66, 67). The *oscillator* strain constitutes a natural *Glra1* null mutation due to a microdeletion in exon 8 that truncates the α1 subunit after TM3 (68). Notably, GlyR function of the *oscillator* polypeptide can be rescued upon coexpression of a C-terminal fragment containing TM4 (69). This result and complementation experiments with different GlyR and

5-HT$_3$ receptor fragments (46) have revealed an essential role of TM interactions in group I LGIC assembly.

Upon recombinant expression, the GlyRα1 subunit forms channels characterized by short mean open times and fast decay kinetics, as found for glycinergic spontaneous inhibitory postsynaptic currents (sIPSCs) in adult spinal cord neurons (70). In retinas from *oscillator* mice, glycinergic sIPSCs in A-type ganglion cells are strikingly reduced and their kinetics slowed due to *Glra1* inactivation (71). Together, the available data indicate that the GlyRα1 subunit defines receptors that are crucial for fast regulation of both motor and sensory functions.

α2-GlyRs—This GlyR isoform is highly expressed at the embryonic and neonatal stages but postnatally is largely replaced by α1 subunit-containing receptors. Synaptically localized α2 staining has been detected in different adult CNS regions, including the spinal cord, brainstem, midbrain, olfactory bulb, and retina, and corresponds to heteromeric α2β receptors (72). At early developmental stages, GlyRα2 forms homo-oligomeric receptors (73), which are extrasynaptically localized and thought to mediate non-synaptic tonic transmission caused by non-vesicular glycine release and/or spillover from adjacent nerve terminals.

Despite the availability of *Glra2$^{-/-}$* mice (74, 75), the precise physiological roles of GlyRα2 are still largely enigmatic because the knock-out animals are phenotypically normal. Electrophysiological recordings of neonatal GlyRs in spinal neurons (70) and glycinergic sIPSCs in narrow-field amacrine cells of *Glra2$^{-/-}$* mice (74) indicate that α2-GlyRs mediate IPSCs characterized by slow decay kinetics. In *Glra2$^{-/-}$* mice, receptive field center responses in the retinal on and off pathways are impaired (76), and hyperalgesia induced by the injection of zymosan is prolonged compared with wild-type littermates (77). Both its known sites of expression and the available physiological data suggest that, in adult mammals, synaptic GlyRα2 is involved mainly in regulating sensory pathways.

α3-GlyRs—Originally, GlyRα3 was considered a minor adult GlyR isoform with an expression pattern resembling that of GlyRα1 (14, 78). In the spinal cord, GlyRα3 staining is found at synaptic sites in laminae I and II of the dorsal horn, where it

inhibits the propagation of nociceptive signals to higher brain regions and serves as molecular substrate of pain sensitization by the inflammatory mediator prostaglandin E_2 (PGE_2) (79). PGE_2 binds to prostaglandin EP2 receptors and thereby activates protein kinase A, which phosphorylates GlyRα3 and thus down-regulates glycine currents in dorsal horn neurons. In $Glra3^{-/-}$ mice, this down-regulation of glycine inhibition by PGE_2 is abolished, and PGE_2 fails to sensitize pain responses (79). Also, the analgesic effect of cannabinoids in mouse models of chronic inflammatory and neuropathic pain is absent in $Glra3^{-/-}$ animals (80). Hence, GlyRα3 constitutes a promising target for the development of novel drugs for the treatment of chronic pain syndromes.

GlyRα3 immunoreactivity has also been detected in other pathways involved in sensory processing. In retina, GlyRα3 is localized at synapses that are distinct from GlyRα1-containing ones (71). By analyzing $Glra3^{-/-}$ mice, amacrine cells of the AII subtype have been shown to contain synaptic α3-GlyRs, which confer medium-fast kinetics on sIPSCs (74). These receptors regulate the receptive fields of ON-ganglion cells by enhancing the excitatory center response (76). Furthermore, GlyRα3 has been found at inhibitory synapses in the inner ear (81). Together, the available data indicate that like GlyRα2, α3-GlyRs have an important role in sensory information processing.

α4-GlyRs—GlyRα4 is the least understood subtype of the GlyR family; this mainly reflects the low abundance of α4 mRNA and protein in the mammalian CNS. In chicken embryos, GlyRα4 transcripts have been detected in the spinal cord, peripheral ganglia, and male genital ridge (82). In the adult rodent retina, GlyRα4-immunoreactive synapses have been found on displaced ON-cholinergic amacrine cells (71). Upon heterologous expression, α4-GlyRs form channels with pharmacological properties largely resembling those of α1 receptors (82). Notably, in humans, the *GLRA4* gene is a pseudogene. Based on the analysis of sIPSCs in narrow-field amacrine cells of $Glra2^{-/-}$ mice, α4 receptors have been suggested to display ultra-slow decay kinetics (74).

GlyRβ Subunit—The *Glrb* gene encodes the rodent β subunit, which is present three times in adult pentameric α1β-GlyRs (9, 10). By *in situ* hybridization, high levels of GlyRβ mRNA have been detected throughout the embryonic and postnatal CNS (78). However, this widespread transcription of *Glrb* is not reflected in a corresponding abundance of the β protein. A recently generated monoclonal antibody revealed GlyRβ immunoreactivity exclusively at GlyR-positive synapses but not in CNS regions lacking glycinergic neurons, such as the cortex (72). This discrepancy between GlyRβ transcript and protein levels likely reflects a retention and rapid degradation in the endoplasmic reticulum of GlyRβ in the absence of α subunits. Immunostainings of adult mouse retina have confirmed a high extent (>90%) of co-localization of GlyRβ with GlyRα1−3 (72). In the case of GlyRα4, however, immunoreactive synapses that lack GlyRβ staining were found in the inner plexiform layer. Thus, a minor subpopulation of synaptic GlyRs might not be heteromeric.

The functional importance of GlyRβ is underlined by the *spastic* mutation in mice, which produces a phenotype identical to that seen in *spasmodic* animals (83). *Spastic* animals express reduced levels of the major adult GlyR isoform α1β due to an intronic insertion of a LINE-1 element in the *Glrb* gene (84, 85). Causative for the reduced expression is exon skipping resulting from a polymorphism of a splice signal amplified by the LINE-1 insertion (86).

Developmental Changes in GlyR Function and Isoform Expression

In contrast to its hyperpolarizing action on adult neurons, glycine depolarizes motor neurons during embryonic development and around birth (87). Due to high intracellular chloride concentrations at these stages, GlyR channel opening leads to chloride efflux and thereby may induce neuronal firing, although inhibition also may occur as a result of the shunting conductance produced by GlyR activation. A depolarizing excitatory function of GlyRs at early developmental stages may be important for synaptogenesis because GlyR-triggered activation of voltage-gated Ca^{2+} channels appears to be crucial for GlyR clustering at postsynaptic sites (88). Also, it may be essential for the regulation of glutamate release by presynaptic GlyRs that have been detected electrophysiologically in large adult nerve terminals, such as the calyx of Held (89) and hippocampal mossy fiber terminals (90). Postnatally, the neuronal chloride equilibrium potential shifts to negative values due to chloride extrusion upon expression of the K^+/Cl^- cotransporter KCC2 (91). Thus, GlyR currents become hyperpolarizing, *i.e.* inhibitory.

In the spinal cord and brainstem, the developmental change in GlyR function described above is paralleled by changes in subunit composition (92). Embryonic and neonatal GlyRs are thought to be extrasynaptically localized homopentamers of α2 subunits (73), whereas adult synaptic GlyRs are heteromers containing α1 (or other α) and β subunits (7, 20). This change in subunit composition alters the biophysical properties of GlyR currents, resulting in faster decay kinetics and a smaller channel conductance (70, 93). Recombinant α2-GlyRs show slower response kinetics and larger subconductance state distributions than α1β receptors, consistent with the *in vivo* properties of neonatal *versus* adult GlyRs (94). Apparently, the postnatal change in GlyR subunit composition fine-tunes inhibitory transmission by shortening the mean channel open time and accelerating the decay of glycinergic IPSCs.

Mutations in Human GlyR Genes Cause Hyperekplexia

Mutations affecting glycinergic neurotransmission cause the hereditary neuromotor disorder hyperekplexia (HKPX; startle disease). The hallmark symptoms of this genetically heterogeneous disorder are an exaggerated startle response to auditory or tactile stimuli and, particularly in neonates, transient muscle rigidity ("stiff baby syndrome"). In 1993, positional cloning disclosed mutations in the *GLRA1* gene localized on chromosome 5q33.1 (termed the *HKPX1* locus) as a major cause of hyperekplexia (95). In this pioneering study, substitutions of the highly conserved residue Arg-271 within the extracellular loop connecting TM2 and TM3 were found in four families with autosomal dominant hyperekplexia. These substitutions decrease the agonist sensitivity and single-channel conductance of

recombinant GlyRα1 (54, 96). Subsequently, additional dominant and recessive inheritance patterns and compound heterozygosity have been described for other patient families in which various *GLRA1* missense or null mutations have been identified (4, 5, 97). Some of these mutations have been found to affect GlyR intracellular trafficking rather than agonist binding or channel gating (69, 98). Consistent with an important role of the β subunit in postsynaptic GlyR function, mutations in the *GLRB* gene have been associated with HKPX2 (97, 99). A third major form of hyperekplexia of presynaptic origin (HKPX3) is due to mutations in the gene encoding the neuronal glycine transporter GlyT2 (*SLC6A5*) (97).

Recent studies have implicated other GlyR genes, in particular *GLRA3*, in the pathology of other neurological disorders, such as autism, human immunodeficiency virus-associated dementia, generalized epilepsy, and amyotrophic lateral sclerosis (4, 5). Furthermore, autoantibodies against GlyRα1 have been found in patients suffering from progressive encephalomyelitis with rigidity and myoclonus (100). Together, all of these studies underline the importance of proper GlyR function for human health.

Conclusion and Perspectives

Since its purification in 1982, considerable progress has been made in elucidating the structure and pharmacology of the GlyR and in identifying physiological functions of its distinct isoforms. Furthermore, mouse models for different GlyR subunit deficiencies have become available, and the importance of GlyR mutations for the pathogenesis of human neuromotor disease is now well understood. However, many unanswered questions await further investigation, including the precise mechanism of GlyR channel gating, the individual roles of the different GlyR isoforms in various normal and diseased brain regions, and the development of isoform-specific ligands.

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